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EVALUATION OF PHYSICOCHEMICAL, ANTIOXIDANT AND ATR-FTIR PROFILES OF SELECTED *APIS CERANA* HONEYS FROM BODOLAND TERRITORIAL REGION, NORTHEASTERN INDIA

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ABSTRACT
Background: *Apis cerana* is a distinct pollinator species that holds a crucial role in ecosystem management. It is known for facilitating the reproduction of various flowering plants and producing rich-flavoured honey.
Objective: To study the biochemical composition, antioxidant properties and spectral fingerprints of three locally sourced *A. cerana* honey varieties: Mustard, Buckwheat and Multifloral, collected from the Bodoland Territorial Region (BTR), Northeastern India.
Methodology: Exploratory characterization of the honey samples was performed using standard biochemical assays and ATR-FTIR spectroscopy technique.
Results: The analyzed honey samples had low pH levels, indicating a moderately acidic nature. Buckwheat honey exhibited the highest moisture (15.54 ± 0.49 %), protein (1.39 ± 0.27 g/100g), TPC (446.66 ± 1.33 mg $_{GAE}$/100g), TFC (40.20 ± 0.06 mg $_{QE}$/ 100g) and RSA (78.82 ± 0.35 %). FTIR spectra demonstrated prominent peaks associated with water, carbohydrates, phenolics, organic acids and lipids in honey samples.
Conclusion: Results indicate that the biochemical, antioxidant and spectroscopic features of honey samples varied based on their floral origin. Buckwheat honey displayed a relatively higher antioxidant activity with lower sugar concentration. The findings provide insights to support honey authentication and advancement in apiculture practices.

KEYWORDS: *Apis*, Bees, Food safety, Honey, Spectroscopy

INTRODUCTION

Beekeeping (Apiculture) is an important agro-based practice in the Northeastern region of India. It involves managing bee colonies to produce honey, wax and pollen, thereby contributing to pollination services [1]. In general, many regions of India have shared a profound connection with beekeeping practice since ancient times, with people undertaking it as a full-time occupation or hobby to support their livelihood for income generation [2]. Bees make honey by converting floral nectar, plant secretions or the excretion of plant-sucking insects into simple sugars [3]. The importance of honey as an essential food source in the human diet and lifestyle is documented in ancient literature and cave paintings, illustrating the harvest and consumption of wild honey by early hominins [4]. Natural honey is composed of various biomolecules, including sugars, enzymes, water, amino acids, bioactive compounds and minerals [5].

Honey biochemistry is largely determined by the nectar, geographical location and processing techniques [6]. The variations may also be linked to the foraging activity of different honeybees as they travel several kilometres from the hive in search of suitable nectar sources, depending largely on their preferences and accessibility [7]. *Apis cerana*, the Asian honeybee, has an estimated foraging distance of 1.5–2.5 km and shows excellent adaptation to scattered nectar sources, with more frequent foraging trips, higher foraging rates, less sugar consumption and greater longevity than certain closely related species [8]. It is also known for producing high-quality, nutritionally rich honey from diverse botanical sources, owing to its prolonged nectar cycle and ability to produce hive products even during the dearth period [9]. Honey produced by *A. cerena* is utilized in both traditional and contemporary medicine for its beneficial properties, including antioxidant, antimicrobial, anti-inflammatory and wound-healing effects [10]. Thus, the long–standing use of honey by humans is associated with its complex nature, leading to unique nutritional and therapeutic potentials [11]. The presence of complex compounds such as polyphenols, flavonoids and other bioactive constituents in honey is considered beneficial in enhancing human health [12]. Worker bees generally forage on various nectar sources including yellow mustard bloom (*Brassica juncea*), the white-pink flowers of buckwheat (*Fagopyrum esculentum*) and various wildflowers, thus can be classified as mustard, buckwheat and multifloral honey based on their pollen dominance [13].

The classification of honey by botanical and geographical origin is important for evaluating its health benefits and potential contamination. It is generally accomplished by the melissopalynology technique, through microscopic identification of pollen grains in honey with reference to published pollen slides and atlases [14]. The chemical parameters in honey, mainly pH, moisture, protein, phenolic, flavonoids and antioxidants, have been examined across different

varieties to explore their nutritional and commercial relevance [15]. Apart from traditional methods, approaches focusing on the association of physicochemical parameters with specific identification markers in various honeys have emerged [16]. FTIR spectroscopy is a rapid and non-destructive method used for assessment of honey based on the differential vibrational patterns of the functional groups. The Attenuated Total Reflectance (ATR) sampling technology is commonly integrated with FTIR spectroscopy, allowing examination of the samples in either solid or liquid state without the requirement for extensive preparation [17].

Even though honey is considered a livestock product, its supply chain to consumers is unique compared to other agriculture-based food chains as it undergoes very minimal processing [18]. Honey is a complete natural product with wide applications due to its versatile health-promoting properties distinct to bee–flora diversity and specific location [19]. While honey is primarily consumed for its nutritional benefits, consumers are often seen forming strong bonds with the place it is harvested. Generally, consumers' trust is closely linked to the availability of sufficient reliable information about the honey, particularly its nutritional composition on the label, enabling them to make better-informed choices [20]. The study aims to investigate the biochemical and spectral fingerprints of honeys produced by *A. cerana* in the Bodoland Territorial Region (BTR) located within the state of Assam in Northeastern India for assessment of quality, traceability and authenticity.

MATERIALS AND METHODS

Study site

Freshly extracted mustard, buckwheat and multifloral honey were procured from Bodoland Territorial Region (BTR), Northeastern India. BTR, lying within the geographical region of 26°7′12″N to 26°47′50″N latitude and 89°47′40″E to 92°18′30″E longitude, is a prominent agricultural site in Assam, a state near the foothills of the Bhutan–Himalayan ranges. The region is known for its agro-based food products and beekeeping practices, along with traditional skills in wild honey harvesting. Indigenous people in BTR have engaged in apiculture for generations to gather honey and support crop pollination.

Sample Collection

Honey samples were harvested by the beekeepers from apiaries by gently removing the frame, uncapping the honeycomb and squeezing out the honey using the crush and strain method. The collection was done during the peak honey flow period from January 2023 to April 2024, when there is bloom in the nectar source for honeybees to store honey. Following extraction, all samples were poured through the strainer and kept in clean, sterile glass bottles until further analysis (Fig 1).

Fig 1: Honey samples collected from BTR, Assam, India

Determination of physicochemical and antioxidant properties

pH value

For determining the pH of honey samples, 10 g honey was added to 75 mL distilled water and stirred to form a solution. The pH readings of the solutions were recorded using a μ pH System 361pH meter following the method described by the Association of Official Analytical Chemists [21].

Moisture (%)

Estimation of the moisture content was conducted according to the method described by the Association of Official Analytical Chemists [21]. Briefly, 3 g honey was weighed and burned at a constant temperature of 100 °C for 3 hours using a hot air oven. The sample was allowed to cool, and the percentage moisture was calculated as: Moisture Content (%) = [(Fresh Weight−Dry Weight)/Fresh Weight] × 100.

Ash (%)
Determination of ash content was conducted using the incineration method described in the Association of Official Analytical Chemists [21], whereby the porcelain crucibles were washed and oven-dried for 20 mins. The crucibles were pre-weighed, and a 5 g honey sample in a porcelain crucible was incinerated for 2 hours at 500 °C in a muffle furnace. Finally, the crucibles were removed from the furnace, left to cool, and the ash content was calculated as: Ash (%) = (Weight of Ash/Weight of Sample) × 100.

Total Sugar
Sugar was measured using the refractometric analysis technique with a Brix Handheld Refractometer [22]. Initially, honey samples were diluted in water and stirred well to make a 25 % (w/v) honey solution. The amount of total sugar was measured and expressed in g/100g.

Proline Content
Determination of proline was performed using ninhydrin solution for colour complex formation and proline as standard. 0.5 mL honey was mixed with 1 mL 80 % formic acid, followed by the addition of 1 mL ninhydrin solution to the mixture. The solution was shaken for 15 min and left in a hot water bath (15 min). 5 mL 2-propanol (50 %, v/v) was mixed into the solution and absorbance of the honey solution and the blank was measured at 510 nm wavelength [23]. Proline was calculated as: Proline (mg/kg) = $(E_s/E_a) \times (E_1/E_2) \times 80$.

Hydroxymethylfurfural
5-Hydroxymethylfurfural was estimated with the White Spectrophotometric method [24]. 5g honey was treated with the clarifying agent (Carrez solution-I & Carrez solution-II), and the volume was adjusted to 100 mL with distilled water. Next, the solution was filtered and absorbance was measured with a portion mixed with 0.2 % sodium disulfite at wavelengths 284 nm and 336 nm. HMF was calculated as: HMF (mg/kg) = $(A_{284}-A_{336}) \times 149.7 \times 5/W$.

Crude Protein
For determination of protein, the micro-Kjeldahl method consisting of three distinct steps: digestion, distillation and titration was adopted [21]. Organic nitrogen in 0.2 g honey held in a digestion flask was converted to ammonium sulphate by adding 10 mL sulphuric acid in the presence of 3 g mixed catalyst (Potassium sulphate and Copper sulphate) until a greenish colour appeared. When the mixture cooled, 30 mL distilled water was added gradually, and the sample was alkalized with sodium hydroxide. The mixture was distilled into boric acid solution by adding two drops of methyl red and one drop of bromocresol green. The ammonium nitrogen concentration was estimated by titrating against 0.1 N HCL and multiplying by 6.25, a universal conversion factor for protein estimation.

Total Phenolic Content (TPC)
Total phenolic content in honey was estimated by using the Folin-Ciocalteu method [25]. Briefly, 1 mL Folin-Ciocalteu reagent was added to 1 mL honey and shaken till the mixture was properly dissolved. After 3 min, 1 mL 10% Na_2CO_3 solution was added to the mixture and the volume adjusted to 10 mL using distilled water. Consequently, the mixture was left to incubate in the dark for 90 min, followed by measuring the absorbance at a wavelength of 760 nm in a UV-Vis Spectrophotometer (model no. UV-1900i) against the blank. Results were presented as mg gallic acid equivalents (GAEs) per 100g honey sample.

Total Flavonoid Content (TFC)
Determination of flavonoids was conducted using the Aluminium Chloride method [26]. Firstly, 200 µL of the methanolic extract of each honey was suspended in 0.2 mL $NaNO_2$ (5 % w/v), and 0.2 mL $AlCl_3$ was added to the mixture. Then the mixture was neutralized with 2 mL NaOH (1N) solution and the volume adjusted to 5 mL using distilled water. Methanol was used as the solvent in the blank solution. The incubation of the solution was done at room temperature for 15 min and absorbance was taken at 510 nm with UV-Vis Spectrophotometer (Model No: UV-1900i). Flavonoid content was calculated from the Quercetin standard curve and expressed in mg quercetin equivalents (QE)/100g honey.

Radical Scavenging Activity (%)
Radical scavenging activity was determined using the DPPH (2,2–diphenyl–1–picrylhydrazyl) assay [27]. For the preparation of DPPH solution, 4 mg DPPH was dissolved in 100 mL 99% methanol. Furthermore, 1 g of honey extract was suspended in 25 mL methanolic DPPH solution and shaken vigorously until mixed well. The mixture obtained was filtered using filter paper and kept in the dark for 15 min. Consequently, 0.008 g ascorbic acid was added to 8 mL methanol and stored as a standard reference. The serial dilutions at concentrations 10, 20, 40, 60 and 80 µg/mL were prepared and incubated for 20 min at room temperature. Absorbance of the samples at 517 nm wavelength was noted and percentage radical-scavenging calculated as: RSA (%) = [(A−B)/A] × 100, where A = absorbance of control; B = absorbance of sample.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)
The PerkinElmer Spectrum IR Version 10.6.1 was used to obtain infrared spectra for honey samples. The samples were subjected to a mid-infrared wavenumber range of 4000–400 cm-1. Firstly, the spectrum of air was collected under the same conditions as that of the investigated samples. Later, the spectra of the air were subtracted from the spectra of the samples to get the FTIR spectra of honey. 1 mL pure honey was loaded into the ATR crystal core centre, and estimation

was done in triplicate. Averages of the FTIR-ATR spectra for honey samples were calculated, and functional groups in honey were identified [28].

Statistical analysis

All analysis were done in triplicate and results were reported as mean ± standard deviation using Microsoft Excel 2007 software package, Version 2410.

RESULTS AND DISCUSSION

Physicochemical parameters

The pH level is an important parameter that reflects the slightly acidic characteristic of natural honey, resulting from the presence of different organic acids. It can act as a key indicator for discriminating honeys derived from various botanical sources and geographical locations [29]. The pH values of the analyzed honey samples were found within the widely accepted range of 3.4–6.1 [30]. Mustard honey showed the lowest pH value (3.42 ± 0.16) while Buckwheat honey displayed the highest pH (4.16 ± 0.69) (Table 1). Low pH in natural honey is crucial as it contributes to its antimicrobial and wound healing properties, which enhance tissue repair by reducing protease activity on the wound site, increasing oxygen release from haemoglobin and stimulating the activity of fibroblast and macrophage cells [31]. In addition, moisture content represents the stability and susceptibility of honey against factors affecting its shelf-life such as fermentation and granulation [32]. The percentage moisture in samples ranged within the standard limit (max. 20 %). However, the highest moisture was found in Buckwheat honey (15.54 ± 0.49 %) while the lowest was observed in Multifloral honey (11.02 ± 0.48 %). Generally, honey with higher moisture percentage is more prone to fermentation and degradation from the action of certain osmotolerant yeast that thrives in moist conditions like that of honey [33]. In contrast, low moisture in honey has been known to enhance its antimicrobial properties, allowing only a few microorganisms to survive in such an extreme environment [34].

Amongst the analyzed honeys, Mustard honey had the lowest ash content (0.06 ± 0.01 %) while Multifloral honey showed the highest ash content (0.14 ± 0.01 %). Despite its presence in small amounts, ash in honey correlates with its mineral concentration, which ultimately depends on the nectar composition of the predominant floral resources [35]. Moreover, the honey sugars function as a major energy source and constitute an important nutritional component, similar to those found in fruits, as they are easily digestible [36]. The highest sugar content was demonstrated by Mustard honey (79.24 ± 0.33 g/100g); however, Buckwheat honey exhibited the least sugar (68.51 ± 0.26 g/100g). This is in accordance with the fact that honey is a concentrated mixture of sugar components, including simple sugars (dextrose, levulose, glucose, fructose) and at least 22 other complex sugars in water [37]. Nevertheless, sugars in honey can eliminate unpleasant odour from burns and skin ulcers, helping in wound healing, as pathogenic bacteria prefer to use the sugar from the honey as an alternative to amino acids from serum and dead tissue [38].

Proline is the most abundant amino acid in honey and an excellent choice as the standard for evaluating amino acid levels [39]. The proline content in honey samples ranged from 280.50 ± 0.46 to 520.83 ± 0.65 mg/kg. Since proline is a crucial qualitative criterion, it is useful in the prediction of honey ripeness and adulteration; if proline is found in a concentration less than the standard limit, i.e. ≥ 180 mg/kg [39]. 5-hydroxymethylfurfural (HMF) is an organic compound formed from the reducing sugars in honey. Being a neo-forming substance, HMF can be influenced by various factors including the honey extraction, processing and storage conditions. It is thus a suitable marker for evaluating honey quality and authenticity [40]. HMF values in honey samples were within the standard limit, i.e., max. 80 mg/kg. Buckwheat honey showed the lowest HMF content with a mean value (5.18 ± 0.05 mg/kg). Mustard honey had the highest HMF content with a mean value (18.24 ± 0.25 mg/kg). Usually, higher HMF content in honey can signify inadequate storage conditions or excessive heating during honey processing [41]. The protein in honey originates mainly from the salivary and hypopharyngeal glands of worker bees, apart from the enzymatic reaction of bee saliva with plant pollen [42]. The difference in protein content amongst differently sourced honey is related to both its entomological and botanical origin [43]. The highest protein was observed in Buckwheat honey (1.39 ± 0.27 g/100g) while Multifloral honey exhibited the lowest protein content (0.67 ± 0.41 g/100g). Despite low concentration, honey proteins serve as vital bioactive markers for authentication and possess pharmacological properties including antioxidant, anti-inflammatory and anti-microbial capacity [44].

Antioxidant properties

Total phenolic content contributes to the nutritional and antioxidant capacity of honey [45]. Besides other bioactive compounds, phenolic acids in honey impact its overall antioxidant potential, which differs according to its floral source as well as geographical location [46]. Buckwheat honey showed the highest phenolic content (446.66 ± 1.33 mg $_{GAE}$/100g), while the lowest was observed in Multifloral honey (245.55 ± 0.98 mg $_{GAE}$/100g). Amongst all the analyzed samples, Buckwheat honey was the darkest in colour while Multifloral honey was the lightest. It has been suggested that the darker coloured honey has higher phenolic content than light-coloured honey varieties. Many studies have reported that darker-coloured honey usually contains higher phenolics and antioxidant capacity [47,48]. A similar result has been reported from the previous study on six different unifloral honeys, with the dark-coloured honey possessing the highest phenolic content and antioxidant capacity [49]. The highest level of flavonoids was exhibited by Buckwheat honey (40.20 ± 0.06 mg $_{QE}$/100g), with Mustard honey exhibiting the lowest flavonoid content (18.25 ± 0.02 mg $_{QE}$/100g). Flavonoids can also serve as potential markers for determining botanical origin, which are exclusively associated with nectar sources and bee species. However, factors like prolonged storage conditions and processing techniques should be considered they can lead to changes and a decline in the concentration of antioxidant compounds [50]. The DPPH activity of honey samples representing the concentration at which 50 % scavenging of free radicals occurred was determined, and the values are reported in Table 1. The DPPH radical scavenging activity of the analyzed honey variants ranged from 62.46 ± 1.28

% to 78.82 ± 0.35 %. The results suggest that Buckwheat honey had the highest radical scavenging potential compared to other samples. Honey antioxidant capacity is greatly influenced by the amount of phenolic and flavonoid compounds [51,52].

Table 1: Biochemical parameters of *A. cerana* honeys from BTR, Assam, Northeast India

Biochemical parameters	Mustard Honey	Buckwheat Honey	Multifloral Honey
pH (%)	3.42 ± 0.16	4.16 ± 0.69	3.68 ± 0.54
Moisture (%)	14.60 ± 0.35	15.54 ± 0.49	11.02 ± 0.48
Ash (%)	0.06 ± 0.01	0.10 ± 0.06	0.14 ± 0.01
Total Sugar (g/100g)	79.24 ± 0.33	68.51 ± 0.26	70.14 ± 0.02
Proline (mg/kg)	280.50 ± 0.46	412.30 ± 1.58	520.83 ± 0.65
HMF (mg/kg)	18.24 ± 0.25	5.18 ± 0.05	13.51 ± 0.91
Protein (g/100g)	1.11 ± 0.6	1.39 ± 0.27	0.67 ± 0.41
TPC (mg $_{GAE}$/ 100g)	352.21 ± 0.09	446.66 ± 1.33	245.55 ± 0.98
TFC (mg $_{QE}$/ 100g)	18.25 ± 0.02	40.20 ± 0.06	20.72± 0.05
RSA (%)	62.46 ± 1.28	78.82 ± 0.35	66.43 ± 1.13

Results are shown as mean ± standard deviation.

ATR-FTIR Spectroscopy

Honey samples were characterized using ATR-FTIR spectroscopy based on different infrared spectra obtained (Fig 2,3,4). The broad band observed at 3271.48 to 3272.24 cm^{-1} may be due to stretching vibrations from O-H groups in water, carbohydrates and organic acids. Furthermore, the region demonstrated stretching vibrations; specifically, from carboxylic acids and the NH_3 stretching band of amino acids, indicating an area showing stretching vibrations primarily driven by sugars in honey's chemical skeleton [53]. Another peak region around 2931.67 to 2934.83 cm^{-1} in honey samples is vibrations arising from biomolecules including cellulose and lipids; prominently due to C-H stretching in CH_2 and CH_3 groups and NH_3 in free amino acids. The bands around 1643.21 to 1643.98 cm^{-1} are from stretching vibrations, namely C=O in amide I, C=O in fatty acid, C=C in unsaturated lipids, O-H deformation in cellulose and H_2O. Peaks around 1416.50 to 1416.79 cm^{-1} can result from deformation in O-CH and C-C-H of sugar molecules or -OH deformation in the C-OH group [32]. Additional broad bands observed within the range 1345.85 to 1255.96 cm^{-1} may arise due to deformations of O-H, C-O, C-H and C=C, likely corresponding to phenolics in honey [54]. The interesting peaks at 1147.24 cm^{-1} and 1147.64 cm^{-1} were present only in Buckwheat and Multifloral honey, respectively, which may correspond to the C-O and C-O-C group vibrations [55]. A further intense band within 1051.73 to 918.27 cm^{-1} may result from stretching in C-O of the C-OH group or C-C in the carbohydrate structure [56]. Peaks lying in the terminal region around 866.14 to 776.42 cm^{-1} may be attributed to vibrations in the anomeric region of the sugar molecules [57].

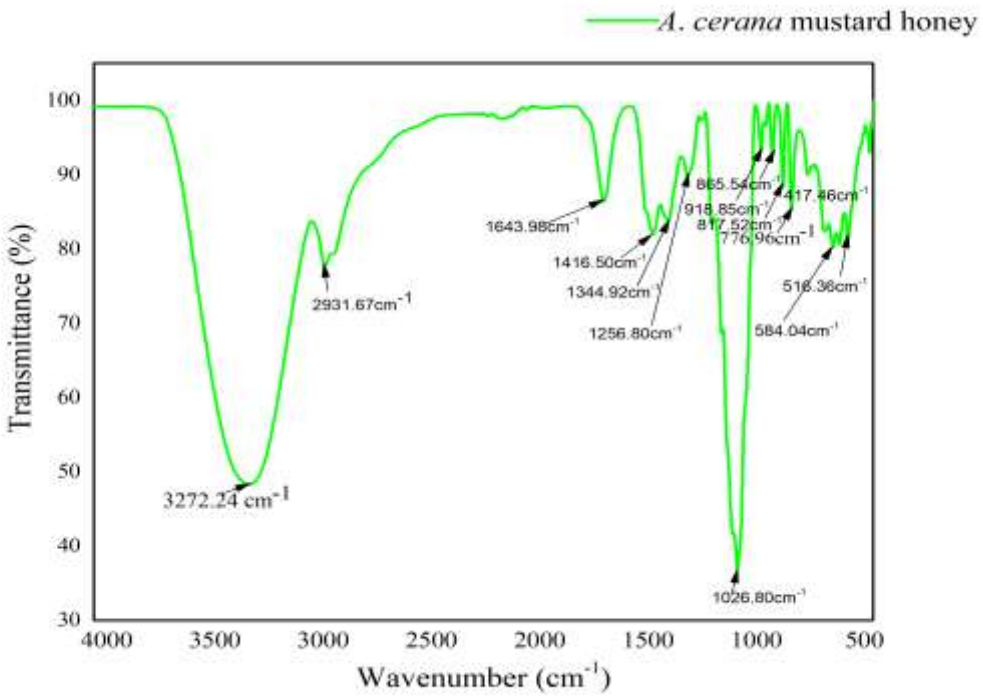

Fig 2: FTIR spectrum of Mustard honey

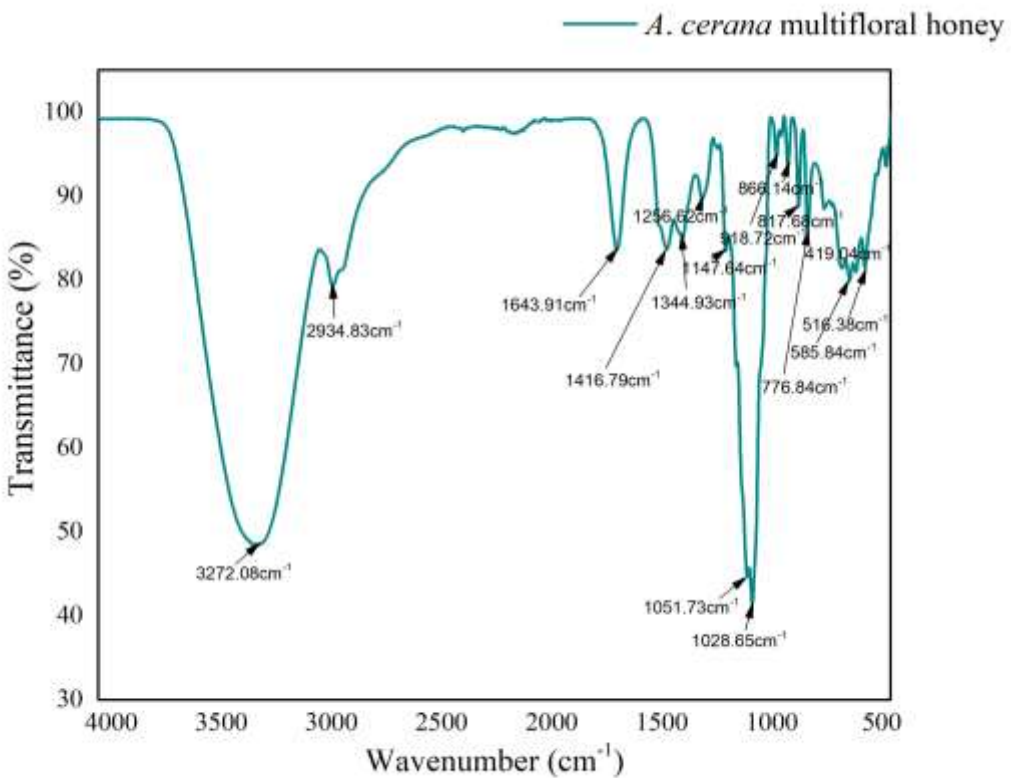

Fig 3: FTIR spectrum of Multifloral honey

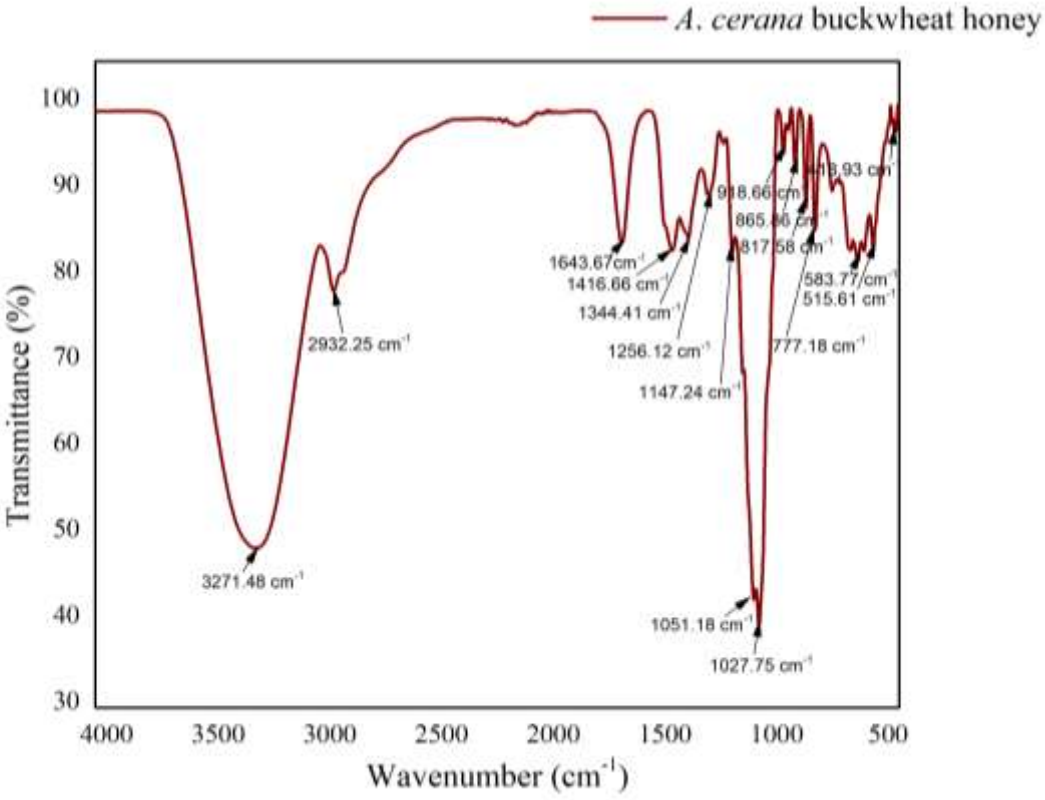

Fig 4: FTIR spectrum of Buckwheat honey

CONCLUSION

A. cerana honey samples from BTR, Northeastern India were evaluated based on variations in their physicochemical parameters, antioxidant capacity and ATR-FTIR spectral patterns. All the honey samples were slightly acidic with parameters lying within the established standard limits. Among the samples, the highest sugar content was demonstrated by Mustard honey, whereas the Buckwheat honey showed the lowest sugar content. Buckwheat honey exhibited the lowest HMF value but demonstrated high protein, TPC, TFC and Radical Scavenging activity. ATR-FTIR Spectroscopy revealed

that although the overall spectral features of the honey samples were nearly comparable, variations were observed in the characteristic absorbance peaks. Notably, the results showed distinct peaks in Buckwheat honey that were absent in other variants. The findings indicate that different honeys can be differentiated and authenticated depending on varying biochemical composition and infrared spectral properties. The results provide valuable insights that will help in authenticating differently sourced honeys and support further intensive studies.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
1. Haldhar SM, Nidhi CN, Singh KI, Devi AS. Honeybees diversity, pollination, entrepreneurship and beekeeping scenario in NEH region of India. Journal of Agriculture and Ecology, 2021:12:27-43.
2. Kishan Tej M, Aruna R, Mishra G, Srinivasan MR. Beekeeping in India. In: Omkar (eds) Industrial Entomology, 2017: (pp. 35-66): Springer Singapore. https://doi.org/10.1007/978-981-10-3304-9_3
3. Codex Alimentarius, International Food Standards. Standard for Honey. CXS12-1981[1]. Adopted in 1981. Revised in 1987, 2001. Amended in 2019, 2022.
4. Crittenden AN. The importance of honey consumption in human evolution. Food and Foodways, 2011: 19(4):257-73.
5. Rodriguez I, Camara-Martos F, Flores JM, Serrano S. Spanish avocado (Persea americana Mill.) honey: Authentication based on its composition criteria, mineral content and sensory attributes. Lwt, 2019:111:561-72.
6. Fechner DC, Hidalgo MJ, Díaz JD, Gil RA, Pellerano RG. Geographical origin authentication of honey produced in Argentina. Food Bioscience, 2020:33:100483.
7. Somanathan H, Warrant EJ, Borges RM, Wallen R, Kelber A. Resolution and sensitivity of the eyes of the Asian honeybees Apis florea, Apis cerana and Apis dorsata. Journal of Experimental Biology, 2009: 212(15):2448-53.
8. Zhang C, Pokhrel S, Wu Z, Miao X, Huang ZY, Yang W. Longevity, food consumption, and foraging performance of *Apis cerana* and *Apis mellifera* in mixed colonies. Apidologie, 2019: 50(2):153-62.
9. Guo J, Ding Q, Zhang Z, Zhang Y, He J, Yang Z, Zhou P, Gong X. Evaluation of the antioxidant activities and phenolic profile of Shennongjia *Apis cerana* honey through a comparison with *Apis mellifera* honey in China. Molecules, 2023: 28(7):3270.
10. Wang Y, Gou X, Yue T, Ren R, Zhao H, He L, Liu C, Cao W. Evaluation of physicochemical properties of Qinling Apis cerana honey and the antimicrobial activity of the extract against *Salmonella Typhimurium* LT2 in vitro and in vivo. Food Chemistry, 2021:337:127774.
11. Kumar D, Hazra K, Prasad PV, Bulleddu R. Honey: an important nutrient and adjuvant for maintenance of health and management of diseases. Journal of Ethnic Foods, 2024: 11(1):19.
12. Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. Contribution of honey in nutrition and human health: a review. Mediterranean journal of Nutrition and Metabolism, 2010: 3(1):15-23.
13. Chauhan DS, Negi GC, Joshi R. Effect of Honey Bee (*Apis cerana*) Dominated Insect Pollination on Yield and Quality of Mustard, Buckwheat and Plum in a Central Himalayan Agro-Ecosystem. International Journal of Ecology and Environmental Sciences, 2024:50(4):543-56.
14. Selvaraju K, Vikram P, Soon JM, Krishnan KT, Mohammed A. Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. Journal of food science and technology, 2019: 56(5):2508-21.
15. Bergamo G, Seraglio SK, Gonzaga LV, Fett R, Costa AC. Physicochemical characteristics of bracatinga honeydew honey and blossom honey produced in the state of Santa Catarina: An approach to honey differentiation. Food Research International, 2019:116:745-54.
16. Bergamo G, Seraglio SK, Gonzaga LV, Fett R, de Mello Castanho Amboni RD, Dias CO, Costa AC. Differentiation of honeydew honeys and blossom honeys: a new model based on colour parameters. Journal of food science and technology, 2019: 56(5):2771-7.
17. Ismail NF, Maulidiani M, Omar S, Zulkifli MF, Radzi MN, Ismail N, Jusoh AZ, Roowi S, Yew WM, Rudiyanto R, Ismail WI. Classification of stingless bee honey based on species, dehumidification process and geographical origins using physicochemical and ATR-FTIR chemometric approach. Journal of Food Composition and Analysis, 2021: 104:104126.
18. Madhwal Y, Pieber E, Yanovich Y, Yakushkina T. Smart contract based honey production supply chain. InProceedings of the 2022 5th International Conference on Blockchain Technology and Applications, 2022: pp. 70-76.
19. Bareke T, Roba K, Addi A. Diversity of bee floral resources and honey production calendar in Ethiopia's southwest shoa zone. Advances in Agriculture, 2024: 2024(1):5428576.
20. Roman A, Popiela-Pleban E, Kozak M. Factors influencing consumer behavior relating to the purchasing of honey part 1. The buying process and the level of consumption. Journal of Apicultural Science, 2013:57(2):159.
21. AOAC International (2005). Official methods of analysis of AOAC International (18th ed.). AOAC International, Gaithersburg MD, USA.

22. Moniruzzaman M, Khalil MI, Sulaiman SA, Gan SH. Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. BMC complementary and alternative medicine, 2013:13(1):43.
23. Kamal MM, Rashid MH, Mondal SC, El Taj HF, Jung C. Physicochemical and microbiological characteristics of honey obtained through sugar feeding of bees. Journal of Food Science and Technology, 2019:56(4):2267-77.
24. Maeda IC, Sampaio AN, Flores Caron EF, Nardy JF, Oliveira SC, Pereira JG, Martins OA. Spectrophotometry of Winkler and White's official methods for the determination of hydroxymethylfurfural in bee honey. Brazilian Journal of Food Technology, 2023: 26:e2022133.
25. Kc Y, Parajuli A, Khatri BB, Shiwakoti LD. Phytochemicals and quality of green and black teas from different clones of tea plant. Journal of Food Quality, 2020: 2020(1):8874271.
26. Salamatullah AM, Alkaltham MS, Hayat K. Effect of microwave roasting on the chemical constituents and antioxidant potentials of coffee beans. International Food Research Journal, 2022: 29(3):552-60.
27. Rahman MM, Abdullah AT, Sharif M, Jahan S, Kabir MA, Motalab M, Khan TA. Relative evaluation of in-vitro antioxidant potential and phenolic constituents by HPLC-DAD of Brassica vegetables extracted in different solvents. Heliyon, 2022: 8(10): e10838.
28. Bong ZR, Shah RM, Chee XW, Hwang SS, Ginjom IR. Simple and rapid characterization of sarawak stingless bee honey using melissopalynological and ATR-FTIR analysis. Food Analytical Methods, 2024:17(5):773-86.
29. Da Silva PM, Gauche C, Gonzaga LV, Costa AC, Fett R. Honey: Chemical composition, stability and authenticity. Food chemistry, 2016:196:309-23.
30. Nzeh J, Quansah L, Dufailu OA. Physicochemical properties of imported and locally produced honey did not translate into its microbial quality and antibacterial activity. Discover Food, 2022:2(1):24.
31. Almasaudi S. The antibacterial activities of honey. Saudi journal of biological sciences, 2021:28(4):2188-96.
32. Ganaie TA, Masoodi FA, Rather SA, Wani SM. Physicochemical, antioxidant and FTIR-ATR spectroscopy evaluation of Kashmiri honeys as food quality traceability and Himalayan brand. Journal of food science and technology, 2021: 58(11):4139-48.
33. Yegge MA, Fauzi NA, Talip BA, Jaafar MB, Othman MB, Yaacob M, Ilyas MA, Ngajikin NH. Reduction in moisture content of dehumidified and microwave-heated stingless bee (Kelulut) honey and its quality. Materials Today: Proceedings, 2021:42:75-9.
34. Prica N, Zivkov-Balos M, Jaksic S, Mihaljev Z, Kartalovic B, Babic J, Savic S. Moisture and acidity as indicators of the quality of honey originating from Vojvodina region. Archives of veterinary medicine, 2015:7(2):99-109.
35. Felsner ML, Cano CB, Bruns RE, Watanabe HM, Almeida-Muradian LB, Matos JD. Characterization of monofloral honeys by ash contents through a hierarchical design. Journal of Food Composition and Analysis, 2004:17(6):737-47.
36. Rahman MM, Richardson A, Sofian-Azirun M. Antibacterial activity of propolis and honey against Staphylococcus aureus and Escherichia coli. African Journal of Microbiology Research, 2010:4(18):1872-1878.
37. Abeshu MA, Geleta B. Medicinal uses of honey. Biology and Medicine, 2016: 8(2):1000276.
38. Kumar KS, Bhowmik D, Biswajit C, Chandira MR. Medicinal uses and health benefits of honey: an overview. J. Chem. Pharm. Res, 2010:2(1):385-95.
39. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food chemistry, 2005:91(3):571-7.
40. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chemistry central journal, 2018:12(1):35.
41. Khalil MI, Sulaiman SA, Gan SH. High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. Food and chemical toxicology, 2010:48(8-9):2388-92.
42. Chua LS, Lee JY, Chan GF. Honey protein extraction and determination by mass spectrometry. Analytical and bioanalytical chemistry, 2013:405(10):3063-74.
43. Bocian A, Buczkowicz J, Jaromin M, Hus KK, Legáth J. An effective method of isolating honey proteins. Molecules, 2019:24(13):2399.
44. Chua LS, Lee JY, Chan GF. Characterization of the proteins in honey. Analytical letters, 2015:48(4):697-709.
45. Pauliuc D, Dranca F, Oroian M. Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication. Foods, 2020:9(3):306.
46. Zawawi N, Chong PJ, Mohd Tom NN, Saiful Anuar NS, Mohammad SM, Ismail N, Jusoh AZ. Establishing relationship between vitamins, total phenolic and total flavonoid content and antioxidant activities in various honey types. Molecules, 2021:26(15):4399.
47. Akbulut M, Ozcan MM, Çoklar H. Evaluation of antioxidant activity, phenolic, mineral contents and some physicochemical properties of several pine honeys collected from Western Anatolia. International journal of food sciences and nutrition, 2009: 60(7):577-89.
48. Can Z, Yildiz O, Sahin H, Turumtay EA, Silici S, Kolayli S. An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. Food chemistry, 2015:180:133-41.
49. Kus PM, Congiu F, Teper D, Sroka Z, Jerković I, Tuberoso CI. Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. LWT-Food Science and Technology, 2014:55(1):124-30.
50. Saric G, Vahcic N, Bursac Kovacevic D, Putnik P. The changes of flavonoids in honey during storage. Processes, 2020:8(8):943.
51. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Analytica chimica acta, 2005:533(2):185-91.

52. Trisha S, Mortuza MG, Rana J, Islam KH, Ferdoush Z, Antora RA, Akash SI, Aziz MG, Uddin MB. Evaluation of the physicochemical qualities and antioxidant properties of some Bangladeshi varieties of honey: A comparative study. Journal of Agriculture and Food Research, 2023:14:100837.
53. Samborska K, Jedlinska A, Wiktor A, Derewiaka D, Wołosiak R, Matwijczuk A, Jamróz W, Skwarczyńska-Maj K, Kiełczewski D, Błażowski Ł, Tułodziecki M. The effect of low-temperature spray drying with dehumidified air on phenolic compounds, antioxidant activity, and aroma compounds of rapeseed honey powders. Food and Bioprocess Technology, 2019:12(6):919-32.
54. Tahir HE, Xiaobo Z, Zhihua L, Jiyong S, Zhai X, Wang S, Mariod AA. Rapid prediction of phenolic compounds and antioxidant activity of Sudanese honey using Raman and Fourier transform infrared (FT-IR) spectroscopy. Food Chemistry, 2017: 226:202-11.
55. Kozłowicz K, Różyło R, Gładyszewska B, Matwijczuk A, Gładyszewski G, Chocyk D, Samborska K, Piekut J, Smolewska M. Identification of sugars and phenolic compounds in honey powders with the use of GC–MS, FTIR spectroscopy, and X-ray diffraction. Scientific Reports, 2020:10(1):16269.
56. Tewari J, Irudayaraj J. Quantification of saccharides in multiple floral honeys using fourier transform infrared microattenuated total reflectance spectroscopy. Journal of agricultural and food chemistry, 2004:52(11):3237-43.
57. Kasprzyk I, Depciuch J, Grabek-Lejko D, Parlinska-Wojtan M. FTIR-ATR spectroscopy of pollen and honey as a tool for unifloral honey authentication. The case study of rape honey. Food Control, 2018:84:33-40.